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## **Comprehensive Genomic Profiling of Carcinoma of Unknown Primary Origin: Retrospective Molecular Classification Considering the CUPISCO Study Design**

Ross, Jeffrey S ; Sokol, Ethan S ; Moch, Holger ; Mileschkin, Linda ; Baciarello, Giulia ; Losa, Ferran ;  
Berlinger, Andreas ; Thomas, Marlene ; Elvin, Julia ; Ngo, Nhu ; Jin, Dexter X ; Krämer, Alwin

**Abstract:** BACKGROUND Carcinoma of unknown primary origin (CUP) accounts for 2%-5% of newly diagnosed advanced malignancies, with chemotherapy as the standard of care. CUPISCO (NCT03498521) is an ongoing randomized trial using comprehensive genomic profiling (CGP) to assign patients with CUP to targeted or immunotherapy treatment arms based on genomic profiling. We performed a retrospective analysis of CUP cases referred for CGP to determine how many were potentially eligible for enrollment into an experimental CUPISCO arm. MATERIALS AND METHODS Centrally reviewed adenocarcinoma and undifferentiated CUP specimens in the FoundationOne database were analyzed using the hybrid capture-based FoundationOne CDx assay (mean coverage, >600×). Presence of genomic alterations, microsatellite instability (MSI), tumor mutational burden (TMB), genomic loss of heterozygosity (gLOH), and programmed death-ligand 1 (PD-L1) positivity were determined. RESULTS A total of 96 of 303 patients (31.7%) could be matched to an experimental CUPISCO arm. Key genomic alterations included ERBB2 (7.3%), PIK3CA (6.3%), NF1 (5.6%), NF2 (4.6%), BRAF (4.3%), IDH1 (3.3%), PTEN, FGFR2, EGFR (3.6% each), MET (4.3%), CDK6 (3.0%), FBXW7, CDK4 (2.3% each), IDH2, RET, ROS1, NTRK (1.0% each), and ALK (0.7%). Median TMB was 3.75 mutations per megabyte of DNA; 34 patients (11.6%) had a TMB greater or equal to 16 mutations per megabyte. Three patients (1%) had high MSI, and 42 (14%) displayed high PD-L1 expression (tumor proportion score ≥50%). gLOH could be assessed in 199 of 303 specimens; 19.6% had a score of ≥16%. CONCLUSIONS Thirty-two percent of patients would have been eligible for targeted therapy in CUPISCO. Future studies, including additional biomarkers such as PD-L1 positivity and gLOH, may identify a greater proportion potentially benefiting from CGP-informed treatment. Clinical trial identification number. NCT03498521 IMPLICATIONS FOR PRACTICE: The findings of this retrospective analysis of carcinoma of unknown primary origin (CUP) cases validate the experimental treatment arms being used in the CUPISCO study (NCT03498521), an ongoing randomized trial using comprehensive genomic profiling to assign patients with CUP to targeted or immunotherapy treatment arms based on the presence of pathogenic genomic alterations. The findings also suggest that future studies including additional biomarkers and treatment arms, like programmed death-ligand 1 positivity and genomic loss of heterozygosity, may identify a greater proportion of patients with CUP potentially benefiting from comprehensive genomic profiling-informed treatment.

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# Comprehensive Genomic Profiling of Carcinoma of Unknown Primary Origin: Retrospective Molecular Classification Considering the CUPISCO Study Design

JEFFREY S. ROSS<sup>1,2</sup>, ETHAN S. SOKOL<sup>1</sup>, HOLGER MOCH<sup>3</sup>, LINDA MILESHKIN<sup>4</sup>, GIULIA BACIARELLO<sup>5</sup>, FERRAN LOSA<sup>6</sup>, ANDREAS BERINGER<sup>7</sup>, MARLENE THOMAS<sup>8</sup>, JULIA ELVIN<sup>9</sup>, NHU NGO<sup>10</sup>, DEXTER X. JIN<sup>11</sup>, ALWIN KRÄMER<sup>12</sup>

<sup>1</sup>Foundation Medicine, Inc., Cambridge, Massachusetts, USA; <sup>2</sup>SUNY Upstate Medical University, Syracuse, New York, USA; <sup>3</sup>University of Zurich and University Hospital Zurich, Zurich, Switzerland; <sup>4</sup>Peter MacCallum Cancer Centre, Melbourne, VIC, Australia; <sup>5</sup>Gustave Roussy, Villejuif, France; <sup>6</sup>Hospital de Sant Joan Despí Moisès Broggi, Barcelona, Spain; <sup>7</sup>F. Hoffmann-La Roche Ltd, Basel, Switzerland; and <sup>8</sup>German Cancer Research Center (DKFZ) and University of Heidelberg, Heidelberg, Germany

Disclosures of potential conflicts of interest may be found at the end of this article.

**Key Words.** Unknown primary tumors • Genetic profiling • Loss of heterozygosity • Molecular targeted therapy

## ABSTRACT

**Background.** Carcinoma of unknown primary origin (CUP) accounts for 2%–5% of newly diagnosed advanced malignancies, with chemotherapy as the standard of care. CUPISCO (NCT03498521) is an ongoing randomized trial using comprehensive genomic profiling (CGP) to assign patients with CUP to targeted or immunotherapy treatment arms based on genomic profiling. We performed a retrospective analysis of CUP cases referred for CGP to determine how many were potentially eligible for enrollment into an experimental CUPISCO arm.

**Materials and Methods.** Centrally reviewed adenocarcinoma and undifferentiated CUP specimens in the FoundationCore database were analyzed using the hybrid capture-based FoundationOne CDx assay (mean coverage, >600×). Presence of genomic alterations, microsatellite instability (MSI), tumor mutational burden (TMB), genomic loss of heterozygosity (gLOH), and programmed death-ligand 1 (PD-L1) positivity were determined.

**Results.** A total of 96 of 303 patients (31.7%) could be matched to an experimental CUPISCO arm. Key genomic alterations included *ERBB2* (7.3%), *PIK3CA* (6.3%), *NF1* (5.6%), *NF2* (4.6%), *BRAF* (4.3%), *IDH1* (3.3%), *PTEN*, *FGFR2*, *EGFR* (3.6% each), *MET* (4.3%), *CDK6* (3.0%), *FBXW7*, *CDK4* (2.3% each), *IDH2*, *RET*, *ROS1*, *NTRK* (1.0% each), and *ALK* (0.7%). Median TMB was 3.75 mutations per megabyte of DNA; 34 patients (11.6%) had a TMB greater or equal to 16 mutations per megabyte. Three patients (1%) had high MSI, and 42 (14%) displayed high PD-L1 expression (tumor proportion score ≥50%). gLOH could be assessed in 199 or 303 specimens; 19.6% had a score of >16%.

**Conclusions.** Thirty-two percent of patients would have been eligible for targeted therapy in CUPISCO. Future studies, including additional biomarkers such as PD-L1 positivity and gLOH, may identify a greater proportion potentially benefiting from CGP-informed treatment. *Clinical trial identification number.* NCT03498521 *The Oncologist* 2020;9999:• •

**Implications for Practice:** The findings of this retrospective analysis of carcinoma of unknown primary origin (CUP) cases validate the experimental treatment arms being used in the CUPISCO study (NCT03498521), an ongoing randomized trial using comprehensive genomic profiling to assign patients with CUP to targeted or immunotherapy treatment arms based on the presence of pathogenic genomic alterations. The findings also suggest that future studies including additional biomarkers and treatment arms, like programmed death-ligand 1 positivity and genomic loss of heterozygosity, may identify a greater proportion of patients with CUP potentially benefiting from comprehensive genomic profiling-informed treatment.

Correspondence: Jeffrey S. Ross, M.D., Foundation Medicine, Inc., 150 Second Street, Cambridge, Massachusetts 02141, USA. Telephone: 617-418-2200; e-mail: jross@foundationmedicine.com Received July 22, 2020; accepted for publication October 28, 2020. <http://dx.doi.org/10.1002/onco.13597>

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## INTRODUCTION

“Carcinoma of unknown primary origin” (CUP) describes a heterogeneous group of cancers determined to be metastatic at diagnosis but for which a primary tumor cannot be identified based on a full standardized diagnostic workup [1]. They are surprisingly common, accounting for 2%–5% of all malignancies, and are associated with extremely poor survival of approximately 1 year or less [2, 3]. Standard therapy for CUP has not changed for decades, a fact that establishes the disease as an unmet medical need requiring immediate attention.

Depending on the clinical constellation, histology, and immunophenotype, CUP can be divided into two clinicopathologic subtypes: the more localized form with a favorable prognosis of 12–36 months, and the widely disseminated form with an unfavorable prognosis of less than 1 year [4]. However, only 10%–15% of patients compose the favorable subset; the majority belong to the poor-risk subset of patients who are treated with platinum-based chemotherapy [4]. Comprehensive genomic profiling (CGP) may reveal more personalized and effective therapeutic options for these patients. Rather than conducting a potentially futile diagnostic search for the primary tumor origin through multiple investigations, including multimodality diagnostic imaging procedures, tissue immunohistochemistry (IHC) panels, serum tumor marker panels, and messenger RNA profiling [5], CGP aims to identify pathogenic genomic alterations in patients with CUP regardless of the primary tumor site [6]. Recent studies have shown a lack of clinical benefit of site-specific chemotherapy or targeted therapies directed by gene expression profiling to determine the tissue of origin (vs. chemotherapy) in patients with CUP [7, 8]; in contrast, evidence for the validity of CGP-informed therapy was bolstered by a study in 2015, in which next-generation sequencing of tumoral DNA from 200 CUP specimens identified at least one clinically relevant genetic aberration in 85% of cases [5]. Notably, one patient with brain metastases harbored an amplification of the *MET* gene and demonstrated a complete clinical response to crizotinib [5]. The efficacy of CGP-informed therapy was further suggested in the recent I-PREDICT trial; in patients with refractory tumors, targeting a larger fraction of identified molecular alterations correlated with significantly improved disease control rates and longer progression-free (PFS) and overall survival (OS) rates [9].

CUPISCO is a phase II, randomized, multicenter study of patients with newly diagnosed, unfavorable CUP (NCT03498521) that will compare the efficacy and safety of targeted therapy or cancer immunotherapy, guided by genomic profiling, with platinum-based standard chemotherapy [10]. All enrolled patients will receive genomic profiling from Foundation Medicine, Inc. on tissue or blood, and, after three rounds of induction chemotherapy, patients experiencing disease control (partial or complete response or stable disease) will be randomized to either standard chemotherapy continuation or experimental treatment of molecularly guided therapies following assignment by a molecular tumor board (Fig. 1). Patients not responding to induction chemotherapy will also undergo molecular tumor

board-based treatment assignment for the same molecularly guided therapies, but in a nonrandomized fashion and without a comparator. Patients will be treated until loss of clinical benefit and will be monitored for PFS (primary endpoint), OS, clinical benefit duration, and safety (secondary endpoints). Results will provide insight into whether CGP-informed therapies are superior to standard unspecific chemotherapy in CUP [10].

The aim of the present study was to perform a retrospective analysis of CUP cases referred to CGP testing at a Clinical Laboratory Improvement Amendments (CLIA)–certified, College of American Pathologists (CAP)–accredited laboratory (Foundation Medicine, Inc.) to estimate how many patients could be matched to one of nine experimental CUPISCO arms based on the inclusion criteria used in CUPISCO. In addition, we aimed to determine whether biomarkers not currently included in CUPISCO, such as programmed death-ligand 1 (PD-L1) status or genomic loss of heterozygosity (gLOH), may provide additional clinical value to CUPISCO and any related future trials. We also examined whether additional mutations not currently used for stratification in CUPISCO may increase the spectrum of patients who can be treated with CGP-informed therapy.

## MATERIALS AND METHODS

Tumor samples were composed of archival tissue from 303 consecutive centrally reviewed adenocarcinoma and undifferentiated CUP cases in the FoundationCore database. CUP was defined as a heterogeneous group of metastatic tumors for which a standardized diagnostic workup fails to identify the site of origin at the time of diagnosis. Criteria to classify as CUP and method of review of patient specimens can be found in the 2015 CUP European Society for Medical Oncology guidelines [11]. Genomic profiling was performed in a CLIA-certified, CAP-accredited laboratory (Foundation Medicine, Inc., Cambridge, MA) using the Illumina HiSeq 4000 instrument (Illumina, Inc., San Diego, CA) on the CDx, U.S. Food and Drug Administration (FDA)–approved platform [12]. At least 50 ng of DNA per specimen was isolated and sequenced to high, uniform coverage (mean, >600×), as previously described [13]. The DNA extracted from CUP formalin-fixed, paraffin-embedded tumor specimens was analyzed after hybridization capture of 324 cancer-related genes and introns from 34 genes commonly rearranged in cancer. Genomic alterations detected by this assay included base substitutions, insertions and deletions (short variants), rearrangements, and copy number changes. Microsatellite instability (MSI), tumor mutational burden (TMB), and gLOH (defined as a biomarker of homologous recombination deficiency and response to poly-ADP ribose polymerase inhibitors [PARPi]) [14] were also calculated, as described previously [15–18].

Regarding TMB, patients were stratified into either TMB-high (TMB  $\geq 16$  mutations per Mb [Mut/Mb]) or TMB-low (TMB <16 Mut/Mb) based on cutoffs used in CUPISCO to determine whether those of the TMB-low cohort, typically associated with a reduced response to immunotherapy [19], could still be matched to a targeted treatment arm. PD-L1 expression was measured by DAKO 22C3 IHC (Dako

Denmark, Glostrup, Denmark) and reported as negative (0% tumor cell staining), low positive (1%–49%), or high positive ( $\geq 50\%$ ). Cases determined by IHC to be *TTF-1+*, *CK7-/CK20+/CDX2+*, or *TPR22:ERG+* were excluded as such tumors belong to a subgroup of CUP with favorable prognosis (lung, colorectal, or prostate cancer). Overlap of biomarkers was also analyzed (gLOH-high, TMB-high, PD-L1-high, MSI-high). Figure 2 presents a summary of the experimental procedure. Approval for this study, including a waiver of informed consent and a Health Insurance Portability and Accountability Act waiver of authorization, was obtained from the Western Institutional Review Board (Protocol No. 20152817).

Sequence analysis methods and validation of the CGP platform used in this study have been described previously by Frampton and colleagues [13, 20]. Base substitution detection was performed using the Bayesian methodology, which enables the detection of novel somatic mutations at low mutant allele frequency (MAF) and increased sensitivity for mutations at hot-spot sites through the incorporation of tissue-specific prior expectations [13]. Reads with mapping quality less than 25 were discarded, as were base calls with quality 2 or less. Final calls were made at MAF of at least 5% (MAF  $\geq 1\%$  at hot spots) to avoid false-positive calls [13], after filtering for strand bias (Fisher test,  $p < .001$ ), read location bias (Kolmogorov–Smirnov test,  $p < .001$ ), and presence in two or more normal controls. To detect short insertions or deletions (indels), de novo local assembly in each targeted exon was performed using the De Bruijn approach [13]. After read pairs were collected and decomposed, the statistical support for competing haplotypes was evaluated and candidate indels were aligned against the reference genome. Filtering of indel candidates was carried out as described for base substitutions [13]. Gene amplifications and homozygous deletions were detected by comparing complete chromosomal copy number maps to reference process-matched, normal control samples, and gene fusions and rearrangements were detected by analysis of chimeric read pairs [13]. Trinucleotide mutational signatures were generated based on techniques described previously [21]. Analysis required the presence of 20 point mutations (excluding pathogenic mutations but including synonymous and noncoding alterations); the mismatch repair signature included COSMIC signature 1 in addition to 6, 15, 20, and 26 [22].

Analysis of germline variants was limited to known or likely variants (no variants of uncertain significance were included). The investigational method for detection of germline mutations, as described previously [23], was demonstrated in 30 tumor samples with matched-normal as a gold standard. In this data set, we observed a 99% accuracy for germline calls (151 of 153 variant calls).

### Statistical Analysis

Error bars on frequency represent the 95% binomial confidence interval. Proportions were compared using the Fisher's exact test. gLOH distributions were compared using the nonparametric Mann-Whitney *U* test.

### RESULTS

Three hundred and three patients were identified who were referred for testing between 2018 and 2019: 96 (31.7%) matched to one of the experimental CUPISCO trial arms (Table 1). The sex ratio was 1:1 (male,  $n = 151$ ; female,  $n = 152$ ), and median patient age was 67 (range, 22–89+) years.

Overall, there were 220 of 324 genes in the FoundationOne CDx bait set that were altered in at least one of the 303 patients (Fig. 3). Key genomic alterations included *ERBB2* (7.3%), *PIK3CA* (6.3%), *NF1* (5.6%), *NF2* (4.6%), *BRAF* (4.3%), *IDH1* (3.3%), *PTEN* (3.6%), *FGFR2* (3.6%), *EGFR* (3.6%), *MET* (4.3%), *CDK6* (3.0%), *FBXW7* (2.3%), *CDK4* (2.3%), *IDH2* (1.0%), *RET* (1.0%), *ROS1* (1.0%), *NTRK1* (1.0%), and *ALK* (0.7%). Of 11 *FGFR2* genomic alterations, 7 (63.6%) were gene fusions or rearrangements. *KRAS* was mutated in 27.4% of specimens, and 6.3% had *G12C* alterations.

Of the 303 samples, 294 had a TMB estimate. Median TMB was 3.75 Mut/Mb of DNA; 23.1% ( $n = 68$ ) of specimens possessed  $\geq 10$  Mut/Mb. Thirty-four specimens (11.6%) harbored  $\geq 16$  Mut/Mb of DNA, and 25 (8.5%) had  $\geq 20$  Mut/Mb.

Of the 34 cases with a high TMB, 23 were assessable for a mutational signature. Fifteen had a dominant mutational signature; eight (34.7%) had a tobacco, five (21.7%) had an ultraviolet light, and two (8.7%) had a mismatch repair mutational signature. Within the TMB-low data set, 252 of 260 (96.9%) specimens displayed pathogenic mutations, with 20 genes altered in at least 5% of specimens; in contrast, within the TMB-high data set, all 34 specimens displayed pathogenic mutations, and 55 genes were altered in at least 5% of specimens (Fig. 4). Out of the 260 TMB-low cases, we observed 18 (6.9%) with *ERBB2* amplifications, 15 (5.8%) with *KRAS G12C* alterations, 7 (2.7%) with *FGFR2* rearrangements, and 5 (1.9%) with *ERBB2*-activating short variants (Fig. 4). Across all cases, alterations in *STK11*, *KEAP1*, and *SMARCA4* occurred in 55 (18.2%), 25 (8.3%), and 34 (11.2%) patients, respectively (Fig. 4); *KEAP1* and *SMARCA4* alterations occurred more frequently in the TMB-high subgroup (*STK11*: 6/34 [17.7%] vs. 49/260 [18.9%]; *KEAP1*: 6/34 [17.7%] vs. 19/260 [7.3%]; *SMARCA4*: 6/34 [17.7%] vs. 26/260 [10.0%]).

The present study also examined whether biomarker analysis not currently included in CUPISCO (such as PD-L1 status, presence of germline mutations, or gLOH) could add further clinical value to the trial.

PD-L1 immunostaining, a predictor of response to immunotherapy, identified 42 cases (13.9%) that displayed a high level of expression of PD-L1. Of the 303 specimens, three (1.0%) were MSI-high. Analysis of putative cancer-associated germline mutations in somatic tumor tissue was limited to 264 samples, with 59 (22.4%) specimens harboring a predicted germline event.

gLOH could be assessed in 199 of 303 specimens, 39 (19.6%) of which had a high gLOH (gLOH  $> 16$ ). Strong homologous recombination-associated genes, including *BRCA1/2* and *PALB2*, were mutated in 11 of 199 (5.5%) cases, and weaker homologous recombination-associated



genes (*RAD51B*, *RAD51D*, *BARD1*, *RAD51C*, *PPP2R2A*, *BRIP1*, *FANCL*, *CDK12*, *CHEK1*, *ATM*, *CHEK2*, and *RAD54L*) were mutated in 18 (9.0%). Mutations in strong homologous recombination-associated genes were associated with a higher gLOH than homologous recombination wildtype (55% vs. 16% had gLOH >16; 95% confidence interval [CI], 23.4–83.3;  $p = .03$ ). Mutations in weak homologous recombination-associated genes were also associated, but not significantly, with a high gLOH (39% vs. 16% had gLOH >16; 95% CI, 17.3–64.3;  $p = .06$ ). Biallelic alterations were more strongly associated with gLOH.

Analysis of overlap of biomarkers (gLOH-high, TMB-high, PD-L1-high, MSI-high) was limited to cases in which the status of all four biomarkers was known ( $n = 191$ ); analysis demonstrated little overlap (Fig. 5).

## DISCUSSION

CUP is among the 10 most common cancers for men and women worldwide [24]. Current treatment strategies, composed of platinum-based chemotherapy, only control the disease for a short period, with most patients surviving less than 1 year after diagnosis [2–4, 8]. CUPISCO is a randomized phase II trial examining the efficacy and safety of CGP-informed targeted therapy and immunotherapy in patients with newly diagnosed CUP [10]. The present study does not include data from CUPISCO itself but is instead a retrospective analysis of CUP cases using the same CGP assay to be used in CUPISCO. The aim was to determine how many cases would be potentially eligible for the targeted therapy and immunotherapy arms of CUPISCO and to inform new arms that could be added to the trial based on the emerging molecular insights. However, it should be noted that the pending data from CUPISCO mean that all findings should be treated with caution and require full validation once CUPISCO commences.

In the study, specimens from 303 patients with unfavorable CUP were analyzed, with 96 (31.7%) being matched to a CUPISCO arm, thus validating the experimental arms used in the study [10]. Key genomic alterations included *ERBB2*, *PIK3CA*, *NF1*, *NF2*, *BRAF*, *PTEN*, *EGFR*, *CDK6*, *BRCA2*, *FBXW7*, *BRCA1*, *CDK4*, *ROS1*, *RET*, *IDH2*, *ALK*, *PTCH1*, and *AKT1*; many of these alterations are potentially actionable with targeted treatment. In that regard, previous studies have shown that carcinomas driven by activating *ERBB2* mutations can respond to anti-*ERBB2* therapies including trastuzumab, lapatinib, and afatinib [25, 26]. Furthermore, in a recent next-generation sequencing-based study of patients with CUP, one patient harboring *ERBB2* amplification was treated with trastuzumab plus paclitaxel and consequently demonstrated a sustained partial response at 9 months until data cutoff [27]. Regarding *BRAF* alterations, a multicohort “basket” study of the *BRAF* inhibitor vemurafenib in patients with nonmelanoma *BRAF*<sup>V600</sup> mutation-positive solid tumors demonstrated clinical responses in 13 unique cancer types, including historically treatment-refractory tumors such as cholangiocarcinoma, sarcoma, glioma, neuroendocrine carcinoma, and salivary gland carcinoma [28]. These results suggest that even single-agent *BRAF* inhibition may have therapeutic

relevance across many cancer types, including CUP [28], although the exact therapeutic regimen may vary depending on the cancer type (e.g., use of *BRAF* inhibitors alone or in combination with other therapies in melanoma and non-small cell lung cancer, respectively [29, 30]).

Interestingly, patients in the present study also displayed genetic changes in the *IDH1*, *MET*, and *FGFR2* genes, as well as *KRAS* G12C alterations. *IDH1* mutations have been found to occur frequently in cholangiocarcinoma which is a putative primary site in many cases of CUP [31]. A phase III trial comparing the *IDH1* inhibitor ivosidenib with placebo in patients with advanced or metastatic mutant *IDH1* cholangiocarcinoma demonstrated a significant improvement in median PFS and, when adjusted for patients crossing from placebo to ivosidenib, OS (PFS, 2.7 vs. 1.4 months; hazard ratio, 0.37; 95% confidence interval [CI], 0.25–0.54;  $p < .001$ ; OS: 10.8 vs. 6.0 months; hazard ratio, 0.46;  $p < .001$ ) [32]. Furthermore, in the study by Ross and colleagues in 2015, one patient with an abdominal mass and solitary brain metastasis on imaging and a 16-copy amplification of the *MET* gene showed a complete clinical benefit upon treatment with crizotinib [5]. In addition to this, based on positive efficacy results in the phase II GEOMETRY mono-1 study [33], the highly potent and selective *MET* inhibitor capmatinib has been granted priority review by the FDA for first-line and previously treated patients with locally advanced or metastatic *MET*-mutated non-small cell lung cancer (NSCLC) [34]. The *FGFR2* inhibitor pemigatinib has also recently been granted accelerated approval for patients with previously untreated, locally advanced or metastatic *FGFR2*-mutant cholangiocarcinoma, and the *KRAS* G12C inhibitor AMG-510 is currently being investigated in phase I/II trials of patients with advanced, mutant solid tumors [35, 36]. As a consequence of results here, pemigatinib and possibly ivosidenib will be included as additional targeted therapies in CUPISCO, thus extending the spectrum of patients who can be treated with CGP-informed therapy.

The present study found the median TMB to be 3.75 Mut/Mb of DNA, with 11.6% of patient specimens possessing 16 or more; numerous studies have found an association of high TMB with clinical benefit from checkpoint inhibitors [19]. Additionally, 97% of TMB-low specimens still harbored pathogenic alterations and would potentially remain eligible for targeted therapy, with notable examples including *ERBB2* amplification, *KRAS* G12C alterations, and *FGFR2* rearrangements. Interestingly, among TMB-high specimens, *STK11* and *KEAP1* were each altered in 6 of 34 (17.7%). Given the recent findings demonstrating that *STK11/KEAP1* mutations reduce the clinical benefit of PD-L1 inhibitors in patients with NSCLC [37, 38], the detection of these alterations in our study may help to inform the use of cancer immunotherapy. *SMARCA4* variants also occurred in 6 of 34 (17.7%) patients, although such alterations have been observed to be enriched in thyroid transcription factor-1 IHC-negative NSCLC [39], suggesting that these specimens may represent NSCLC not detected by the computed tomography scan.

In the present study, 19.6% of specimens had a gLOH score of greater than 16% of the genome, a level that

predicted benefit of the PARPi rucaparib in patients with advanced high-grade epithelial ovarian cancer [17]. Although CUPISCO already includes a PARPi-based treatment arm (olaparib), the high gLOH >16 percentage reported here suggests that gLOH could be used additionally for stratification into this arm. Furthermore, 14% of specimens in the present study displayed a high level of expression of PD-L1 (tumor proportion score  $\geq 50\%$ ), a level which has previously been associated with immunotherapy responsiveness in lung cancer [40]. These findings suggest that use of additional biomarkers such as gLOH and PD-L1 positivity in future studies may identify a greater proportion of patients with CUP potentially benefiting from CGP-informed treatment (up to 19% and 14%, respectively), especially considering the little overlap of PD-L1 positivity or gLOH with TMB-high. The findings also suggest that it may be useful in future trials based on PD-L1 positivity or gLOH to include an additional arm for patients with a diagnosis of CUP, rather than require all patients to be diagnosed with a tumor of specific primary origin.

The present study lacks detailed clinical data for each specimen, including whether any patients received specialized therapy and subsequently demonstrated therapeutic benefit. To date, large-scale evidence illustrating such a benefit of CGP in patients with CUP, compared with traditional chemotherapy, has yet to be reported. In 2017, a prospective clinical trial evaluating the clinical benefit of high-throughput genomic analysis in patients with advanced and heavily pretreated cancers found approximately one-third of patients had improved outcomes with molecularly guided therapy [41]. However, randomized controlled trials are required to quantify the impact of such an approach in the general population of patients with metastatic cancers, especially in cases without extensive pretreatment. A large meta-analysis of phase II single-agent clinical trials revealed that a personalized strategy matching genomic alterations with available targeted therapies was an independent predictor of improved response rate, disease-free survival, and OS and fewer treatment-related deaths when compared with unmatched chemotherapy regimens in a wide array of tumor types [42]. Additionally, various clinical trials assessing the impact of mutation-specific inhibitors, including those targeting *BRAF*, *ERBB2*, *BRCA1/2*, *RET*, or *NTRK* alterations, have shown efficacy in many cancers other than CUP, including lung carcinomas, colorectal cancers, papillary thyroid cancers, and other solid tumors [43–47].

Importantly, in contrast to a mutation-matched therapy approach, a recent phase II trial and the phase II GEFCAPI 04 trial of patients with CUP assessed the efficacy of site-specific chemotherapy directed by gene expression profiling to determine the tissue of origin, versus nonspecific platinum-based chemotherapy; no significant improvement in 1-year survival rate, OS, or PFS was demonstrated [7, 8]. Gene expression-based tissue of origin determination in isolation therefore failed as a strategy to improve the prognosis of patients with unfavorable CUP, further emphasizing the need for novel, primary site-independent treatment options. These negative data support the premise of CUPISCO, which, in contrast to tissue of origin-based conventional chemotherapy, will investigate efficacy of CGP-

informed targeted therapy irrespective of the primary tissue site [10]. In addition, a clinical tumor board based on a central pathology review with an expanded immunohistological marker panel will ensure that only CUP cases with unfavorable outcome are included in CUPISCO. The study presented here was a successful proof of concept for CUPISCO and identified additional biomarkers that may be a target for treatment in patients with CUP. It should be noted that, despite the negative data regarding site-specific treatment based on origin determination by gene expression in patients with CUP, ongoing CUP trials still require identification of a primary tumor origin to avoid including patients without CUP. CGP used here may therefore help to identify these primary tumor origins and thus improve trial enrollment.

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## CONCLUSIONS

The results of the present study showed that approximately 32% of patients with studied CUP specimens would have been eligible for molecularly guided therapy in CUPISCO, thus validating the experimental arms included in the study. Genomic profiling also suggested that additional biomarkers, such as PD-L1 positivity and gLOH, may be useful in future studies to identify a greater proportion of patients with CUP potentially benefiting from CGP-informed treatment. These results provide much needed insight into the therapeutic application of CGP, an approach that will hopefully help to improve the poor prognosis of patients with CUP.

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These analyses have been presented in part as Ross J, Sokol E, Mileskin L et al. Comprehensive genomic profiling (CGP) of carcinoma of unknown primary origin (CUP): Retrospective molecular classification of potentially eligible patients (pts) for targeted or immunotherapy treatment considering the prospective CUPISCO study design. Poster presentation at the European Society for Medical Oncology (ESMO) 2019 Congress, September 27–October 1, 2019; Barcelona, Spain.

## AUTHOR CONTRIBUTIONS

**Conception/design:** Jeffrey S. Ross, Ethan S. Sokol, Holger Moch, Giulia Baciarello, Ferran Losa, Andreas Beringer, Marlene Thomas, Julia Elvin, Alwin Krämer

**Collection and/or interpretation of data:** Jeffrey S. Ross, Ethan S. Sokol, Holger Moch, Linda Mileschkin, Giulia Baciarello, Ferran Losa, Andreas Beringer, Marlene Thomas, Julia Elvin, Nhu Ngo, Dexter X. Jin, Alwin Krämer

**Statistical analysis/quality control of data and algorithms:** Jeffrey S. Ross, Ethan S. Sokol, Andreas Beringer, Dexter X. Jin, Alwin Krämer

**Manuscript writing:** Jeffrey S. Ross, Ethan S. Sokol, Andreas Beringer, Ferran Losa, Andreas Beringer, Dexter X. Jin

**Critical revision of the manuscript for important intellectual content:** Jeffrey S. Ross, Ethan S. Sokol, Holger Moch, Linda Mileschkin, Giulia Baciarello, Ferran Losa, Andreas Beringer, Marlene Thomas, Julia Elvin, Nhu Ngo, Dexter X. Jin, Alwin Krämer

**Final approval of the manuscript:** Jeffrey S. Ross, Ethan S. Sokol, Holger Moch, Linda Mileschkin, Giulia Baciarello, Ferran Losa, Andreas Beringer, Marlene Thomas, Julia Elvin, Nhu Ngo, Dexter X. Jin, Alwin Krämer

## DISCLOSURES

**Jeffrey S. Ross:** Foundation Medicine, Inc. (H, OI, Other-leadership role); **Ethan S. Sokol:** F. Hoffmann-La Roche Ltd (OI), Foundation Medicine, Inc. (E); **Holger Moch:** F. Hoffmann-La Roche Ltd,

Definiens (Other-travel/accommodation/expenses), F. Hoffmann-La Roche Ltd, Definiens, Bayer, Ipsen, Amgen (C/A), F. Hoffmann-La Roche Ltd. (RF-Institution); **Linda Mileschkin:** F. Hoffmann-La Roche Ltd, Beigene (Other-travel/accommodation/expenses); **Giulia Baciarello:** F. Hoffmann-La Roche Ltd, Astellas, Janssen Oncology, Sanofi (H), AstraZeneca, Astellas, Janssen Oncology, Ipsen, Sanofi, Amgen (Other-travel/accommodation/expenses), Janssen Oncology, Sanofi, Astellas Pharma, F. Hoffmann-La Roche Ltd, EuroPharma, Simon-Kucher and Partners, Modra Pharmaceuticals (C/A); **Ferran Losa:** F. Hoffmann-La Roche Ltd, Amgen, Merck (RF-Institution), F. Hoffmann-La Roche Ltd, Merck (Other-travel/accommodation/expenses), F. Hoffmann-La Roche Ltd, Amgen, Merck, Sanofi, Servier (C/A), F. Hoffmann-La Roche Ltd, Sanofi (Other-speaker bureau/expert testimonies); **Andreas Beringer:** F. Hoffmann-La Roche Ltd. (E); **Marlene Thomas:** F. Hoffmann-La Roche Ltd. (E, OI, Other-leadership)

**Julia Elvin:** Foundation Medicine, Inc. (OI, Other-leadership role); **Nhu Ngo:** Foundation Medicine, Inc. (E); **Dexter X. Jin:** F. Hoffmann-La Roche Ltd (OI), Foundation Medicine, Inc. (E); **Alwin Krämer:** F. Hoffmann-La Roche Ltd, Daiichi Sankyo, AbbVie (H), F. Hoffmann-La Roche Ltd, Bayer (H-Institution), Bayer, Merck (RF-Institution), F. Hoffmann-La Roche Ltd, Celgene, Daiichi Sankyo (Other-travel/accommodation/expenses), F. Hoffmann-La Roche Ltd, Daiichi Sankyo, Bristol-Myers Squibb, AbbVie (C/A).

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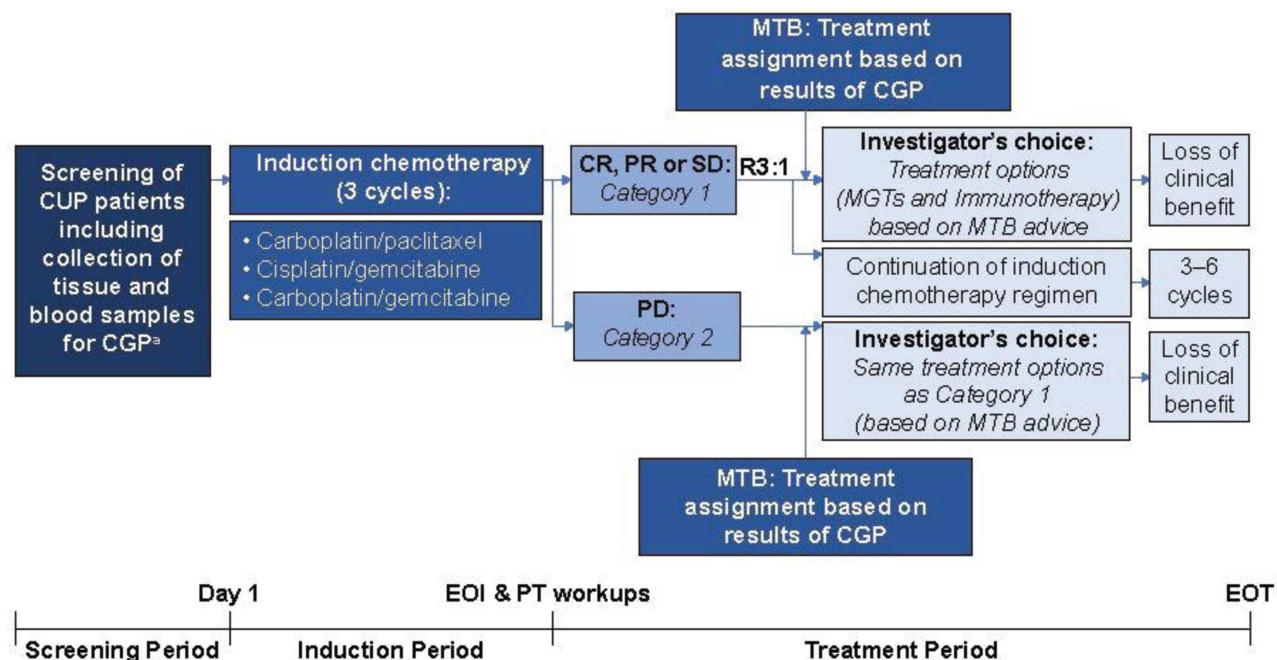
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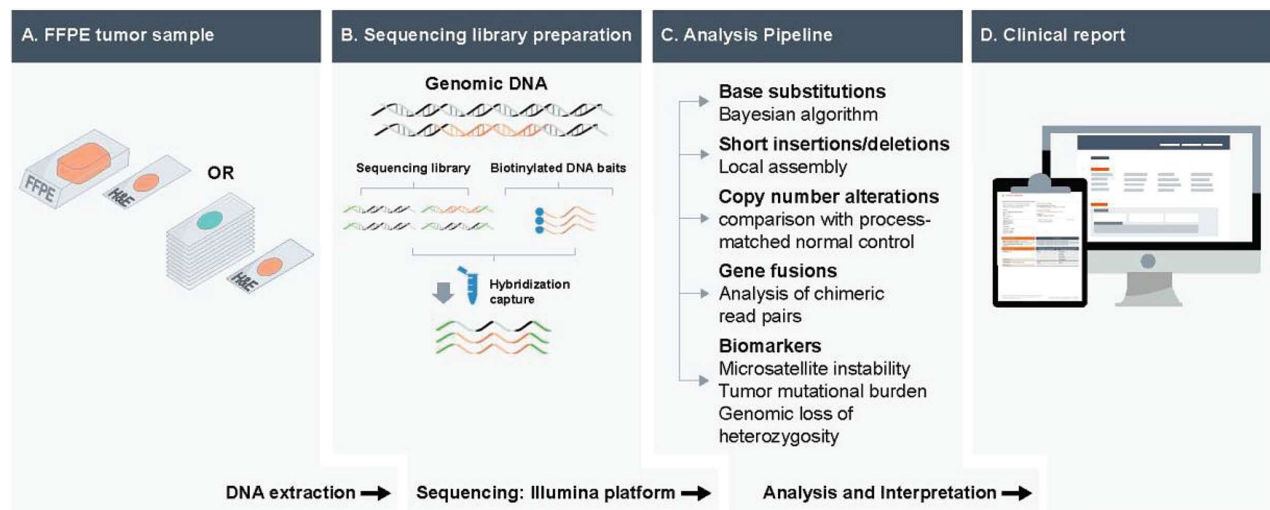
**Table 1.** Genomic alterations and corresponding treatment options in 303 patients with CUP

CUPISCO arm	Genomic alterations (%)
Any targeted therapy	31.7
Subcutaneous trastuzumab + intravenous pertuzumab + intravenous chemotherapy ( <i>ERBB2</i> actionable alterations)	9.0
Atezolizumab (TMB-high [ $\geq 16$ mutations/Mb], MSI-high)	9.0
Ipatasertib plus paclitaxel ( <i>AKT1</i> , <i>PI3K</i> actionable alterations, PTEN loss)	8.0
Olaparib ( <i>BRCA1</i> , <i>BRCA2</i> or select alterations in <i>BRIP1</i> / <i>PALB2</i> )	6.0
Vemurafenib + cobimetinib ( <i>BRAF</i> <sup>V600</sup> alterations)	3.0
Erlotinib + bevacizumab ( <i>EGFR</i> actionable alterations)	2.0
Vismodegib (inactivating <i>PTCH1</i> , activating <i>SMO</i> alterations)	1.0
Alectinib ( <i>ALK</i> , <i>RET</i> rearrangements)	1.0
Entrectinib	0.33



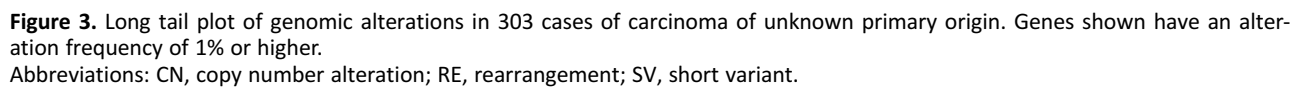
**Figure 1.** CUPISCO study design. <sup>a</sup>Based on eligibility criteria that are summarized at ClinicalTrials.gov: NCT03498521 [46]. Randomization is stratified by gender and response during the induction period (CR + PR vs. SD).

Abbreviations: CGP, comprehensive genomic profiling; CR, complete response; CUP, carcinoma-of-unknown-primary-origin; EOI, end of induction; EOT, end of treatment; MGT, molecularly guided therapy; MTB, molecular tumor board; PD, progressive disease; PR, partial response; PT, pretreatment; R, randomization; SD, stable disease.

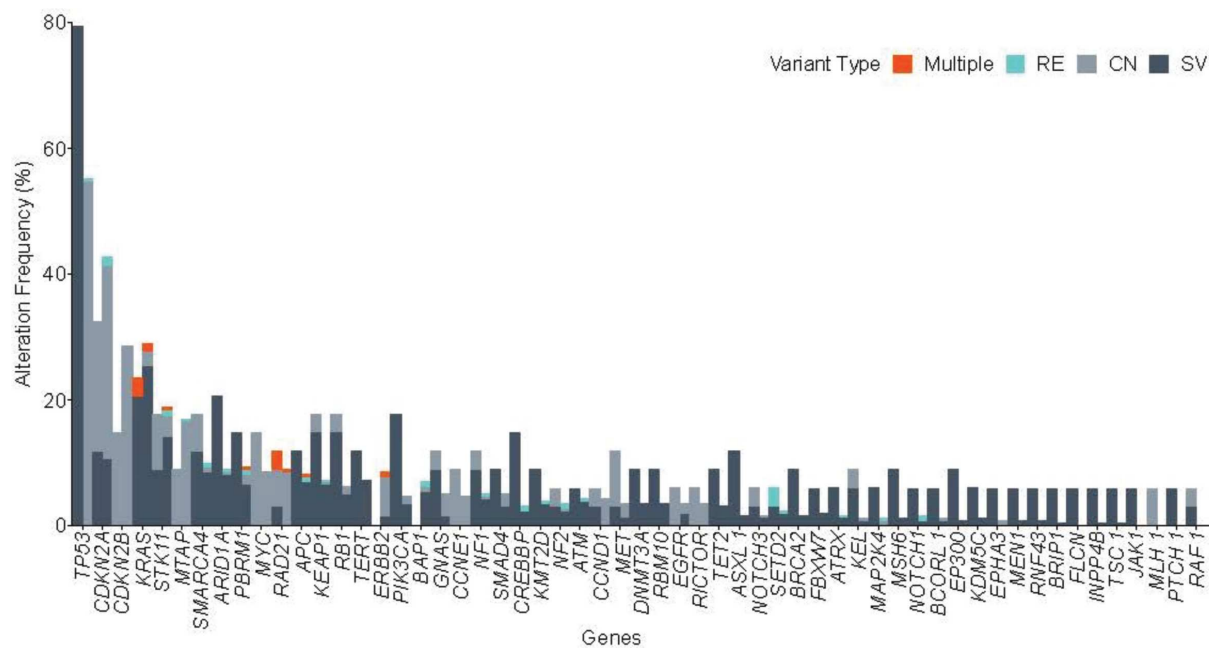


**Figure 2.** Sample, analysis, and report flow. **(A):** FFPE tumor sample. **(A):** Sequencing library preparation. **(C):** Analysis pipeline. **(D):** Clinical report.

Abbreviation: FFPE, formalin-fixed, paraffin-embedded.

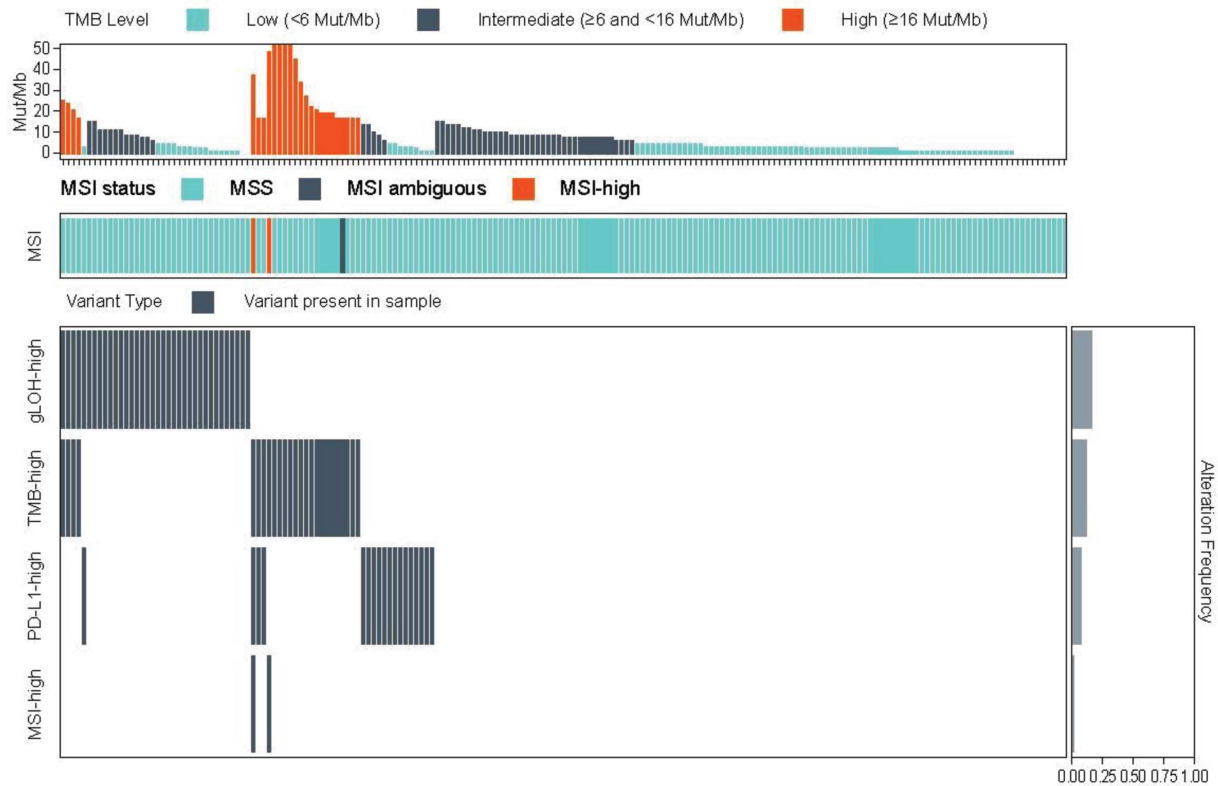






**Figure 4.** Long tail plot of genomic alterations in tumor mutational burden (TMB)-high and TMB-low cases. Genes shown have an alteration frequency of 1% or higher. For each gene, the alteration frequency for TMB-high and TMB-low cases is indicated by the left and right bar, respectively.

Abbreviations: CN, copy number alteration; RE, rearrangement; SV, short variant.



**Figure 5.** Tile plot of overlap of biomarkers gLOH-high, TMB-high, PD-L1-high, and MSI-high. Abbreviations: gLOH, genomic loss of heterozygosity; MSI, microsatellite instability; MSS, microsatellite stable; Mut, mutation; PD-L1, programmed death-ligand 1; TMB, tumor mutational burden.